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PHYTOPATHOLOGY

VOLUME X NUMBER 10

OCTOBER, 1920

WILT OF WHITE CLOVER, DUE TO BRACHYSPORIUM TRIFOLII

LEE BONAR

WITH THREE FIGURES IN THE TEXT

During the summer of 1919 Dr. C. H. Kauffman observed a peculiar diseased condition on a plot of white clover (*Trifolium repens*) on his lawn, at Takoma Park, Washington, D. C. The sod on this lawn was composed almost exclusively of white clover which had been sown on newly cleared land in the spring. The clover seed was bought at a Washington seed store and was used to start the first sod ever grown on this land.

Symptoms of the disease. The first noticeable effect of the disease consisted in the decidedly wilted condition of the attacked clover plants. Later all the leaves and petioles of the plants in an infected area became light brown in color, as well as much shriveled and dry. It seemed to appear in spots, which then spread until these coalesced and the whole of the lawn became a withered brown mass. The disease ran most of its course coincident with continued rainy and muggy weather, the last stages of collapse being furthered by the drying out of the soil and exposure to the sun.

Examination of the diseased clover plants at intervals showed them to be infected by a fungus, and some of the fungus was collected and brought to Ann Arbor. In November, 1919, the work of culturing the causal organism was undertaken. Single spores of the fungus were isolated and grown on artificial media. The fungus grew readily in the laboratory and produced the typical conidiophores which had been found on the diseased clover plants in great numbers.

Inoculation experiments. Pots of clover were grown in the greenhouse, and when the plants were well developed they were sprinkled with a heavy suspension of the spores of the fungus taken from a pure culture, which in turn came from a single spore. These pots were then imme-

diately placed under bell jars, in order to prevent drying out. Uninoculated pots were also placed under bell jars to serve as check. Inoculations were made on clover plants varying in development from fifteen days old, with the third leaf just forming, to those that had well developed prostrate stems and many leaves to each plant and were beginning to form flower stalks.

On the sixth day following the inoculation there appeared on several of the leaves of the more mature plants small spots having a water-soaked appearance. These spots were small at first and appeared on a single leaflet. After a few days, however, they were observed to be spreading to the other two leaflets, and then to affect the petiole also. As the infection spread through the leaf, the spot first formed gradually became light brown in color and somewhat shriveled. At the end of ten days after inoculation, a great many of the leaves and petioles showed infection, and as the infection progressed down the petiole the leaves drooped to the ground and became dry and withered. The infection progressed in the petioles as far as the prostrate stems of the plant, but in no case were the stems observed to be infected. After a short time the stems would put out new leaves, which were subsequently attacked by the fungus when the pots were kept under moist conditions. When, however, the pots were allowed the free air of the greenhouse the infection was not so vigorous, and the plant was able to develop or maintain some green leaves. Photographs of pots 1 and 2, figure 3, show the inoculated plants and checks. Pot 1 and the check plants, pot 2, were photographed one month after inoculation and ten days after removing the bell jars and allowing them to grow in the greenhouse air. Attempts to inoculate the prostrate stems by making incisions with a scalpel, introducing the spores and mycelium of the fungus, and then covering the wound with grafting wax to prevent drying out failed to yield any positive results.

The fungus and the host. The mycelium is stout, septate, and much branched, and ramifies through the tissues of the clover leaves. Within the leaves it is hyaline to pale brown in color, while the aerial mycelium is dark brown. The invasion of the leaf by the fungus causes such a complete collapse of the tissue that the relation of the mycelium to the host cells is made out with difficulty. In so far as could be determined, the mycelium is, for the most part at least, intercellular and there are no haustoria present.

About the time the leaves begin to turn brown there can be seen appearing over the surface of the leaf the upright unbranched conidiophores. These grow out through the epidermis of the leaf, which is by this time in a shrunken and disorganized condition. The conidiophores are dark brown in color, and measure 60 to 90 by 5 microns. Under the hand

lens the growth of these conidiophores and the attached conidia appears as a dark brown, powdery surface in some places on the leaf. The conidia are borne on the tips of the conidiophores, one to several on a single conidiophore, as in figure 1 (*a*). They are brown, 3-septate, and the third cell from the point of attachment is bulged, so that there is a decided hump on one side of the spore, so much so in some cases that the spore is very nearly triangular. For the form of the spore see figure 1 (*b*). On germination the germ tubes are developed from the end cells of the spore (fig. 1 (*c*)).

Besides the spores above described there was found on the inoculated plants another spore form. Occasional small, dark spots could be seen on the leaves that were in the process of going down under the attack of the fungus. These spots were about 1 mm. in diameter, and under the hand lens showed an apparently lighter margin. Microscopic examination of these spots showed them to be a pushing up of the epidermis by a growth of hyphae from beneath, and showed the lighter margin about the spot to be merely the colorless epidermis which had been pushed up by the growth of the fungus from the underlying leaf tissue. This covering soon burst and disappeared, and exposed an acervulus which stood distinctly above the surface of the leaf tissue. The acervulus is composed of branched conidiophores, which form a very dense layer at their tips. This layer is composed of chains of spores, which are cut off in succession. (See figure 2 (*a*), which is a section 10 microns in thickness.) These spores, so formed, are very different from the above described spores (fig. 2 (*c*)). These acervuli were observed on all the inoculated plants, but soon ran their course, and after a very short time only the large, up-standing conidiophores could be found on the material. Neither of these spore forms were ever found on the check plants, and only the large, septate spore form was ever found in culture.

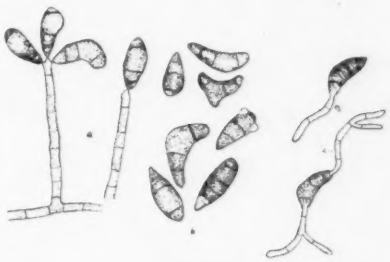

FIG. 1. BRACHYSPORIUM TYPE OF SPORES

(*a*) Conidiophores and mode of attachment of spores; (*b*) form of spores; (*c*) germination of spores (21 to 31 by 9 to 11μ).

The fungus in culture. The fungus was grown on the following culture media: synthetic sugar-peptone agar, corn meal agar, oat meal agar, potato agar, steamed rice, potato plugs, dead stems of *Melilotus alba*, and on dextrose and maltose solutions. The mycelium is generally dark brown in culture, although there is a wide range of the shades of brown in

the different kinds of media used. Spore formation was most abundant on the corn meal and oat meal agars. The greatest number of spores were probably formed on the corn meal agar. On corn meal agar a black felt of aerial mycelium is formed over the surface of the medium, and this is composed of interwoven mycelium and the upright conidiophores. Conidia were also borne on short outgrowths from the horizontal mycelium. These outgrowths were sometimes not more than half the length of the conidia themselves. The agar itself was turned a deep purplish black in color. On oat meal agar, the growth of the aerial mycelium was more marked, as it in some cases practically filled the space under the lid of the petri dish. The medium became at first fawn-colored and then gradually changed to a "deep army brown" (Ridgeway). Potato agar yielded a

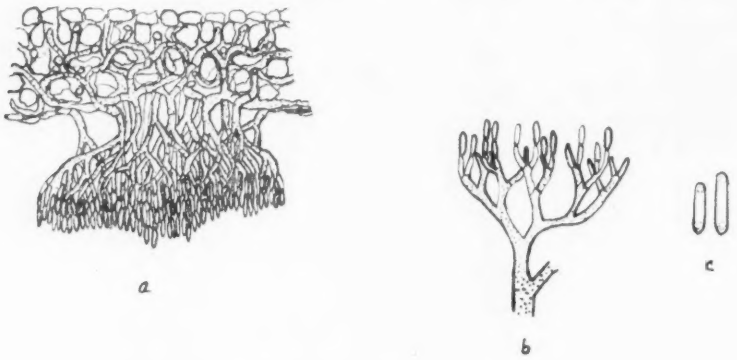

FIG. 2. BLENNORIA TYPE OF SPORES

(*a*) Detailed structure of conidia-bearing hyphae of an acervulus (epidermis has disappeared); (*b*) enlarged portion showing branching and spores in chains; (*c*) single spores. (Section 10μ.)

good vegetative growth, the color changing through a slate-brown to almost jet-black; conidia were very rare in these cultures. On potato plugs the mycelial growth was slow and sparse; no conidia were formed. In maltose solution (2 per cent) there was a growth of blackish mycelium for about ten days, after which growth ceased; no conidia were formed. In dextrose solutions (1 to 5 per cent) the growth was more extensive and continued for one to two months, producing a mat of black mycelium; no conidia were formed. In these solutions irregular clumps of oval to spherical dark-brown cells were developed in the mycelium in great abundance. These were also formed in the various agar mediums. On synthetic-sugar-peptone agar there was a mycelial growth less dense than that on the oat meal and corn meal agars, but there was an abundant formation of conidia on the sugar-peptone agar. On the stems of *Meli-*

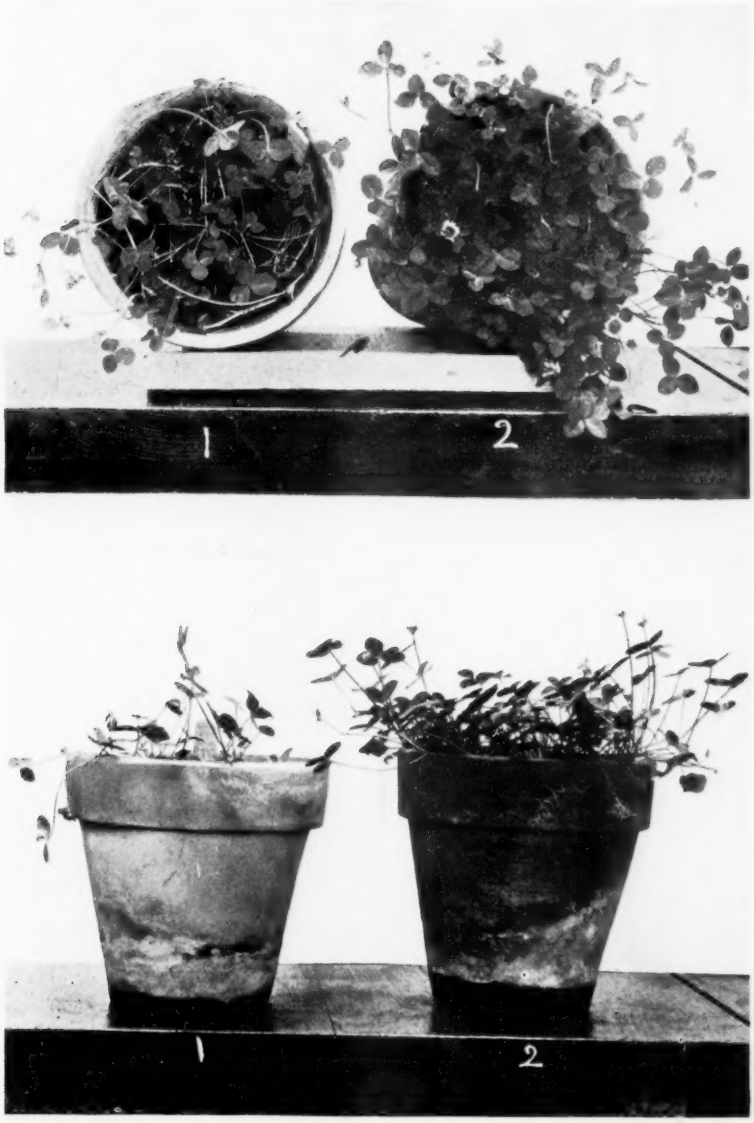

FIG. 3. POTS NO. 1 SHOW EFFECT OF *Brachysporium trifolii* ON WHITE CLOVER ONE MONTH AFTER INOCULATION. POTS NO. 2, CHECK PLANTS

lotus alba a dense outgrowth of conidiophores appeared from all sides of the stem, and on the tips of these a very great number of conidiospores were borne.

Cultures of all of the above named kinds of substrata were kept under observation for five months, under varying conditions as to light and temperature. Test-tube cultures were allowed to dry out slowly so that at the end of five months many of them were air dry. No difference of growth was observed between cultures kept in the light and in the dark. No growth occurred in cultures kept at 4° to 6°C. Slow growth, without the formation of conidia, occurred in cultures kept from 10° to 14°C. for seventy-five days. Growth was rapid and conidia formation abundant in cultures kept at temperatures ranging around 20° to 22°C. No growth occurred in cultures kept at 26°C.

Identity of the fungus.[1] The larger and very common spore-form clearly belongs to the genus *Brachysporium* Sacc. among the Dematiaceae. This genus, as at present limited, contains species whose spores, as found in published exsicatti, certainly do not depart much from the typical Helminthosporium spores. The mere fact that they are characterized as shorter than Helminthosporium spores seems scarcely a sufficient generic distinction; at the same time, other species of the genus Brachysporium possess spores approaching or similar to those of the white clover fungus. It would seem that the marked ventricose or asymmetric characters of certain species, along with limited septation, could be made a better basis of distinction between the two genera. Both contain parasitic species, and a more complete knowledge of their life-histories is desirable for mycologic purposes.

The undoubted presence of another spore-form, produced in an acervulus, raises the question as to the proper position of this species. Since no ascus stage has been matured—although suspected—in culture, the naming of the fungus has to do with the spore forms at hand. Theoretically it might be assumed that the acervulus stage is one of higher standing mycologically; still this would be a mere assumption. The characters of the latter spore form throw it into the genus Blennoria Fr. There can be no question, however, that this stage is of secondary importance in the life history of the fungus. This is shown by its sparse occurrence on the host, by its absence in a variety of cultures, and by the fact that infection occurs readily and abundantly when the Brachysporium spores are used exclusively. At any rate such a name would be of little practical value as compared with the one suggested for it. The following is the technical diagnosis of the fungus as far as it is known.

[1] Identity of the fungus and the technical description by Dr. C. H. Kauffman.

Technical description

Brachysporium trifolii sp. nov. Kauffman. Hyphae rigid, brown, varying to sub-hyaline within the host, or dark fuscous in culture, 4 to 5 microns in diameter, septate, branched; dematiaceous conidiophores arising singly, simple, rigid, stout, strict, dark fuscous, 60 to 90 microns long, 5 to 6 microns in diameter; conidia unequally ventricose-pyriform, mostly curved, varying to obovate, very rarely triangular-lobed, obtuse to rounded above, sub-acute downwards, usually 3 septate, antepenultimate cell enlarged, especially on the convex side, dark fuscous brown, thin-walled and smooth, 21 to 31 by 9 to 11 microns. Secondary sporeform in acervuli on the host, sub-epidermal at first, erumpent, conidiophores crowded, much-branched, upwards sub-hyaline, slender; conidia apical in chains, continuous, hyaline, sub-cylindric, obtuse at the ends, 8 by 10 by 2 to 3 microns.

Parasitic on leaflets and petioles of *Trifolium repens*. Takoma Park, near Washington, D. C.

Conclusions. As a result of the above recorded studies and a comparison of these with the published records of other clover diseases, it appears clear that we have here a distinct and undescribed fungus which is pathogenic to white clover (*Trifolium repens*).

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JUNE 21, 1920

OBSERVATIONS ON CYTOSPORA CHRYSOSPERMA IN THE NORTHWEST

ERNEST E. HUBERT

INTRODUCTION

The weather conditions during the unusually dry summers of 1917, 1918 and 1919 were influential factors in the widespread and abundant appearance of *Cytospora chrysosperma* (Pers.) Fr. upon certain forest, shade and ornamental trees in the Northwest. Observations and experiments have demonstrated that this canker-producing fungus readily enters and parasitizes a host that has previously become weakened or injured or had its twigs or branches killed by drought or other causes. There is, therefore, a real danger of epidemics of this disease resulting from drought.

Long[1] has given a good account of the characteristics of this canker and has drawn special attention to the fact that suppressed, injured or drought-stricken trees are particularly susceptible to the attacks of this fungus.

The fungus is very common throughout the states of Montana, Idaho, Wyoming and Washington, and causes considerable damage to a variety of trees. The following list of collections gives but an indication of its distribution, and includes some new hosts. The numbers refer to the collections in the herbarium at the Laboratory of Forest Pathology, Spokane, Washington. *Cytospora chrysosperma* has been collected and reported from the following places:

On *Populus trichocarpa:* Camp Crook, S. D., No. 6699; Boulder, Mont., Nos. 9204, 9558; Great Falls, Mont., No. 9062; Missoula, Mont., Nos. 647, 3967, 1253; (Propagation cuttings) No. 6696; Twin Lakes, Idaho, reported; Newport, Wash., No. 116; Spokane, Wash., No. 1252.

On *Populus tremuloides:* Deadwood, S. D., No. 11435; Bonner, Mont., reported; Driggs, Ida., No. 87.

On *Populus balsamifera:* Lyman, Wyo., No. 8997; Priest River, Ida., reported.

On *Populus deltoides:* Deadwood, S. D., reported; Missoula, Mont., Nos. 6697, 645; Miles City, Mont., No. 11046.

[1] Long, W. H. An undescribed canker of poplars and willows caused by Cytospora chrysosperma. Jour. Agr. Research **13**: 331–343, May, 1918.

On *Acer circinatum* (scorched by fire); Missoula, Mont., No. 6697.
On *Acer rubrum:* Spokane, Wash., Nos. 10165, 10568.
On *Acer glabrum:* Locke, Wash., reported; Missoula, Mont., 10029.
On *Prunus demissa* (scorched by fire): Missoula, Mont., No. 6072.
On *Sambucus glauca:* Bellingham, Wash., No. 8053.
On *Sorbus scopulina:* Missoula, Mont., reported, No. 3966.
On *Salix lasiandra:* Idaho City, Ida., No. 6703.
On *Salix spp.:* Wheatland, Wyo., No. 9210; Melrose, Mont., No. 11404; Glasgow, Mont., No. 10566; Darby, Mont., No. 6700; Noxon, Mont., No. 10763; Addie, Ida, reported; Priest River, Ida., No. 11808; Twin Lakes, Ida., reported; Spokane, Wash., Nos. 10161, 10162.

SUSCEPTIBILITY OF LOW-VIGOR HOSTS

Inoculations conducted under controlled conditions at a greenhouse at Missoula, Montana, indicate that the fungus is parasitic (wound parasite) when once an entrance is secured through killed or weakened tissues. The following inoculations were made by means of incisions in the bark with a sterile scalpel and spore tendrils from infected material placed within the cut. The inoculated plants were enclosed for five to seven days within celluloid cylinders plugged at both ends with cotton.

No. 6682[2]

Three plants of *Populus trichocarpa* 12 to 15 inches in height were inoculated with pycnospores of *C. chrysosperma* on June 25, 1916. One plant 12 inches in height, with small leaves, was of decidedly low vigor at the time of inoculation; the other two were vigorous (seedlings from natural habitat) as were also the controls. Cankers and small raised pustules were noted on the first plant on September 20, and coiled spore masses were present in abundance on November 25. This plant died. The two vigorous plants failed to become infected. The vigorous plants were seedlings transplanted into the greenhouse the previous season.

No. 5987

Three cuttings of *P. trichocarpa* 16 to 18 inches in height were next inoculated on April 13, 1917. All three plants were noted as dying on October 17. During the month of November a large quantity of pycnospores were produced from typical cankered areas.

[2] Collection number.

No. 5987a

Three cuttings of *P. trichocarpa* 10 to 12 inches in height were inoculated on April 13, 1917, and then placed for nine days in large test tubes lightly plugged, with water enough to supply each cutting. They were then potted in the greenhouse. Abundant pycnospores were noted on November 16, and more were produced following this date. All three of the cuttings died.

No. 6678

Two plants of *P. trichocarpa*, one 15 inches in height, of low vigor due to drought, and one vigorous 17 inches in height, were inoculated April 13, 1917. The weaker plant developed an abundance of pycnidia by September 5, 1917, while the more vigorous plant developed only one small canker with a few pycnidia in October of the same year.

The inoculation experiments show that the low-vigor plants succumbed to the disease more readily, while the notably vigorous plants did not even become infected, or showed only slight infection.

A large number of field observations corroborate the rather meager inoculation data. On June 21, 1917, a young tree of *Sorbus scopulina* about 12 feet in height was found in a yard at Missoula, Montana, which had the upper half of its limbs and twigs killed by *Cytospora chrysosperma* (No. 3966). Inquiry brought out the fact that drought had caused the first signs of low vigor in the tree. This was soon followed by cankered areas on the upper parts of branches and twigs, and finally death of these parts. The disease finally extended to within three feet of the ground. Careful pruning removed the diseased parts, and new sprouts developed from the base the following year. These remained free of disease during 1918. Several other cases also were noted on the same species of tree, and in all of these low vigor was induced by neglect of the trees. J. R. Weir records a set of three large trees of the above named species in a yard in Missoula, Montana, which received insufficient water during the dry summer of 1917. The trees gave evidence of extreme low vigor. *Cytospora chrysosperma* invaded the dead tips of branches and twigs, and in the fall of the same year all three finally succumbed to the girdling effect of the fungus. He also records a heavy infection of the fungus on the shade trees (*Sorbus scopulina*) at Bozeman, Montana, in 1916. The attack was so severe that a large number of the trees were killed in the same year and were later uprooted and destroyed. Many instances are noted in various cities and towns of the Northwest where cottonwood trees used as shade trees contracted the disease after being weakened by neglect or by injuries and were finally killed by it.

On July 22, 1919, five shade trees of *Acer rubrum* growing along the parking on one of the residential streets of Spokane, Washington, were found in a dead or dying condition. Inspection disclosed the tendrils of *Cytospora chrysosperma* issuing from cankered areas on the lower trunk in each case. Injury to the lower trunk and neglect, were determined as the weakening factors. Very few infections were found in the twigs in this case. Sprouts had developed at the base of the dead trees.

Light ground fires developing in the forest often injure and weaken a large variety of the more hardy shrubs and trees. These later develop characteristic cankers, and finally the deep red spore tendrils of *Cytospora chrysosperma* appear. The host attacked is eventually killed by the fungus. *Prunus demissa*, *Sorbus scopulina*, *Acer circinatum*, *Populus tremuloides*, *Populus trichocarpa*, and species of *Salix* are the hosts most commonly affected in this manner. No doubt the list of hosts will be extended as collections increase. A collection on *Quercus* sp. (No. 4837) made in Pennsylvania is also to be mentioned here.

On cut-over areas where slash fires develop several years after the logging operations, an enormous amount of the Cytospora disease is found on the weakened and scorched cottonwoods, willows and maple growing on these areas. In these cases both the scorched surfaces of the trunks and the upper parts of twigs and branches become infected. Such conditions hasten the death of the hosts.

The Cytospora canker has been found to be quite common on almost all species of willow, and an unusual form of infection has been collected on this host (No. 10566). In this case a well-formed witches'-broom had developed by the dying out of the main shoots and the subsequent growth of numerous new shoots behind the infected areas. These shoots in time become infected also. Long[3] has mentioned this advance of the disease from the ends of cut-off branches to the new shoots formed about the pollarded areas.

There are two characteristic forms of pycnospore deposits found upon the bark of the hosts. The most common one is the spore-tendril or spore-horn form, in which the pycnospores issue from the pycnidia in long, coiled, dark red masses. These coils are formed under normal moisture conditions. If, however, abnormal moisture is present but not sufficient to wash away the spores, globular, sticky masses of spores are formed about the openings of the pycnidia. These dry down to hard, dark red hemispherical masses. In some few cases a tendency toward lighter colors is found on certain hosts, ranging from a salmon-orange to orange-chrome.[4]

[3] Loc. cit., p. 332–338.

[4] Ridgeway, Robert. Color standards and color nomenclature. 43 p., 53, col. pl. Washington, D. C., 1912.

AMOUNT OF DAMAGE CAUSED

The damage caused to trees growing in the forest is vastly greater in amount, though less from a financial standpoint, than that caused to shade and ornamental trees, since most of the hosts attacked in the forest are of the broadleaf species, which are not so extensively used in the lumber industry in this region as are the conifers.

Cytospora chrysosperma causes one of the few diseases which are found to damage seriously and eventually kill certain species of shade and ornamental trees. Its habit of attacking trees of low vigor, gradually parasitizing the tissues, and finally killing a portion or all of the tree by girdling, lends itself admirably to the conditions under which a large number of such trees are found. During the dry summer and autumn many of these trees are neglected. Insufficient watering is the most common cause of low vigor, and this results in weakened or killed twigs and branches. Often careless pruning or untreated injuries open a way to the entrance of the fungus. Fire sometimes damages shade and ornamental trees, as well as those in the forest. In nurseries where propagation cuttings of cottonwood and willow are grown the disease is very likely to make its appearance, since under these conditions the fungus readily gains entrance. In the spring of 1917, at Missoula, Montana, about fifty cuttings of *Populus trichocarpa* were planted out in propagating beds by a nurseryman. During the summer every one of the cuttings died. Characteristic cankers were formed near the base of the stems, and upon placing several specimens in damp chambers for one to three days abundant spore tendrils of *Cytospora chrysosperma* were produced (No. 6696). Reports have been received of similar cases in other nurseries. Cuttings of cottonwood shipped into Montana from North Dakota have been found heavily infected by the disease. Care, therefore, should be taken to prevent the spread of the disease through infected nursery stock.

In the Northwestern states, and in Montana particularly, the majority of shade trees planted in the earlier days of the communities were species of Populus. These were selected because they were expected to develop better under the arid and semi-arid conditions of certain localities than some of the other better known or more desirable species. It has been found that the cottonwoods are undesirable for shade trees in parkings, lawns, etc., on account of the damage caused to cement sidewalks and tile piping by the fast-growing and wide-spreading root system. In Missoula, Montana, and in Spokane, Washington, many cement walks have been upheaved and broken by the growth of large roots of cottonwoods beneath them. Cottonwoods are very susceptible to injury

through neglect. Lack of water, mechanical injuries and heavy pollarding or pruning being very common forms of neglect and abuse, the trees are easily attacked and killed by the Cytospora. In recent years fewer cottonwoods are being planted, and the old trees are being replaced by more desirable species. The willows, maples and mountain ash (*Sorbus scopulina*) are attacked to a less extent. Few willows are used for shade or ornamentation, but maples and mountain ash are used extensively in this region. The conditions under which the fungus thrives present a real danger of epidemics developing in years of unusual drought.

CONTROL METHODS

The following control methods are recommended:

a. The most resistant species, or those not attacked by the fungus, should be selected for planting.

b. Shade trees should be watered regularly and abundantly, and should be protected against mechanical injuries.

c. Rigid inspection of nursery stock should be enforced, to prevent the spread of the disease.

d. All infected portions of nursery stock and of infected trees found in parkings, lawns, etc., should be cut away and burned.

OFFICE OF INVESTIGATIONS IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
SPOKANE, WASHINGTON

THE EFFECT OF CITRIC ACID ON THE GERMINATION OF THE TELIOSPORES OF PUCCINIA GRAMINIS TRITICI[1]

A. F. Thiel and Freeman Weiss

With One Figure in the Text

It is generally believed that the teliospores of stem rust will not germinate until spring, following exposure to low temperature and alternate freezing and thawing. Evidence that a natural rest period is required was obtained by failure to secure germination of these spores after subjection to low temperature (−10°C.) and repeated freezing and thawing in early December, 1919.

Means were then sought for shortening the rest period, which it was conceived might be due to either or both of two causes, viz., after-ripening changes in the spore contents or low permeability of the spore wall to water. The following methods of overcoming these conditions were suggested by the work of Crocker (1), Eckerson (3), and Rose (4) on after-ripening in seeds, and of Shull (5) and Denny (2) on the permeability of seed coats.

1. To hasten after-ripening:

a. Subjection to increased oxygen pressure.

b. Treatment with acids to modify the enzymic changes occurring within the spore protoplasm and to increase its hydration capacity.

2. To modify the permeability of the spore wall:

a. Subjection to alternate freezing and thawing and to constant low temperature.

b. Treatment with lipoid solvents and acids to change the chemical nature of the wall.

Spores were treated by the methods outlined under 2, but none of the treatments caused them to germinate. However, when placed in dilute citric acid for short periods, they subsequently germinated readily. The apparently specific stimulating effect of this acid on the germination of stem-rust teliospores is considered of sufficient interest to justify a preliminary report.

[1] Coöperative investigations between the Agricultural Experiment Station of the University of Minnesota and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

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On August 12, 1919, telial material of *Puccinia graminis tritici* was collected at University Farm, St. Paul, Minn., and stored outside in wire cages. Short pieces of rusted straw were exposed to chloroform vapor for one minute. It was found that this exposure to chloroform vapor either killed saprophytic fungi or inhibited their growth. Acidulated alcohol was similarly effective but 95 per cent alcohol alone did not prevent the development of contaminating fungi nor did exposure to ether for thirty seconds. In these cases various molds, particularly Penicillium, Helminthosporium, and Fusarium, developed on about one-third of the test material.

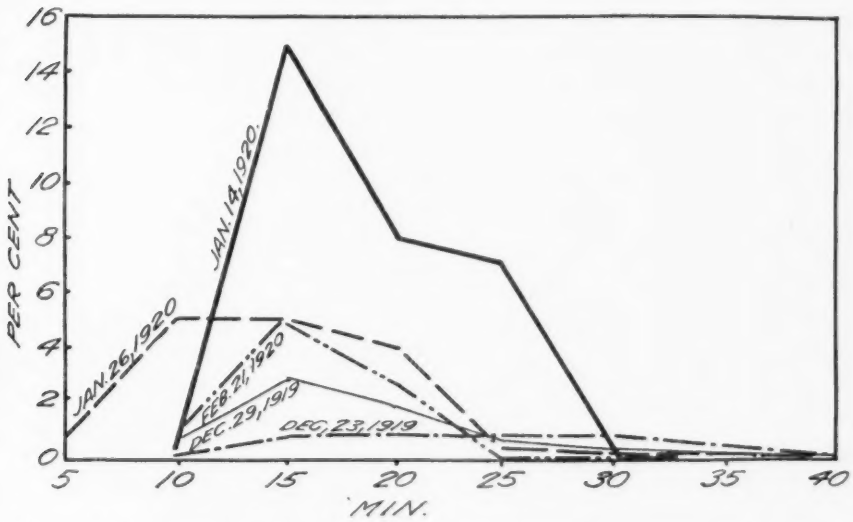

FIG. 1. GRAPHS SHOWING THE PERCENTAGE OF VIABLE TELIOSPORES OF *Puccinia graminis tritici* WHEN TREATED WITH 1 PER CENT CITRIC ACID DURING DIFFERENT PERIODS OF TIME

After exposure to chloroform vapor the stems were immersed for various periods, ranging from five minutes to one hour, in 1 per cent citric acid. The germination tests were then made by placing the treated material in small vials, lined with moist filter paper. These were corked and placed in a refrigerator where the temperature was about 6°C. Experimentation had shown that better germination could be obtained by this method than by any of the hanging-drop methods. The percentage of germination was determined by carefully transferring small portions of the telia to a drop of water on a slide, slightly separating the material, adding a cover slip, and examining under the microscope. This treatment, when carefully carried out, did not result in separation of the promycelia,

and subsequent development could be followed on the slide. Counts were made of the number of germinating spores in three different portions of the field.

Teliospores treated with 1 per cent citric acid were examined for germination on December 23 and 29, 1919, and January 14, January 26, and February 22, 1920, as shown in table 1. Control tests were made on each occasion, but no untreated material germinated at any time. The other treatments listed in table 2 also were not successful in causing germination. The best germination obtained was 15 per cent from teliospores which had been exposed to citric acid for fifteen minutes.

Repeated results indicate that this period of immersion is the optimum (see fig. 1). In one out of five trials 1 per cent of the teliospores germinated after exposure for five minutes and 0.2 per cent germinated after exposure for forty-five minutes. No germination occurred after longer exposure.

TABLE 1

The effect of citric acid on germination of teliospores of Puccinia graminis tritici, as shown by germination percentages of lots treated for periods varying from five to forty-five minutes

DATE ON WHICH EXAMINATION FOR GERMINATION WAS MADE	DURATION OF EXPOSURE IN MINUTES								
	5	10	15	20	25	30	35	40	45
December 23, 1919.....	0	0.3	1	1	1.0	1.0			0.2
December 29, 1919.....	0	0.6	3	2	0.7	0.3	0.15	0	0
January 14, 1920.....	0	0.4	15	8	7.0	0.2	0	0	0
January 26, 1920.....	1	5.0	5	4	0.5	0.1	0	0	0
February 21, 1920.....	0	0.8	5	3	0.2	0	0	0	0

Germination began after two and a half days. A vigorous, well-developed promycelium with completely formed sporidia was produced in three days. In most cases only the lower cell germinated. The upper cell of a few spores sent out a promycelium, but in no case did both cells of a single spore germinate.

On February 25, 1920, teliospores which had been exposed to citric acid for fifteen minutes were placed on leaves of *Berberis vulgaris* and *fremontii* and the plants were then kept in a moist chamber for forty-eight hours. The beginning of pycnial development was clearly visible on both hosts on March 7, and abundant pycnia were formed by March 10. On February 27 inoculations again were made, some with teliospores which had been treated with citric acid and others with spores which had been soaked only in distilled water. Pycnia again appeared in eleven days where treated spores had been used, but no infection was obtained from the checks.

Tests also were made with butyric acid, as this has been shown (3) to be effective in hastening after-ripening of seeds, but inconclusive results were obtained. Germination of a single spore was observed in material that had been stored indoors since its collection in November and not subjected to alternate freezing and thawing. On the other hand, no germination was obtained from material which had been stored outside all winter. Further experiments are under way.

It is highly probable that germination of teliospores might have been obtained much earlier had this treatment been discovered earlier in the season. The nature of the effect of citric acid is at present under investigation, and various salts of the acid are being tested. The fact that

TABLE 2

Treatments used in germination experiments with teliospores of Puccinia graminis tritici, the combination employed being indicated by asterisks. No germination was obtained in any case

CHEMICAL	TREATMENT						TREATED MATERIAL EXPOSED TO		
	Soaked in distilled water one hour placed in solution for number of minutes indicated.						Alternate freezing and thawing	Temperature of 6°C. in ice box.	Temperature of 25°C. in incubator
	10	20	30	40	50	60			
HCL, $\frac{M}{100}$	*		*			*	*	*	*
Phosphoric acid, 1 per cent	*		*			*	*	*	*
H_2SO_4, $\frac{M}{100}$, $\frac{5M}{100}$, $\frac{M}{10}$, and $\frac{2M}{10}$	*	*	*	*	*	*		*	*
Tannic acid, 1, 2, and 5 per cent	*	*	*	*	*	*		*	*
Lactic acid, 1, 2.5 and 5 per cent	*	*	*	*	*	*		*	*
KCL, $\frac{M}{100}$, $\frac{5M}{100}$, $\frac{M}{10}$, and $\frac{2M}{10}$	*	*	*	*	*	*		*	*
Phenol, 1, 2.5 and 5 per cent	*	*	*	*	*	*		*	*
H_2O_2 (Merck's), 0.0625, 0.125, 2.5, and 5 per cent	*	*	*	*	*	*		*	*
Acetic acid, 1, 2.5 and 5 per cent	*	*	*	*	*	*		*	*

lipoid solvents and sodium hydroxide were ineffective, though ordinarily increasing the permeability of cell walls to water, makes it appear doubtful that the effect is wholly on the spore wall. The fact that acids other than citric do not produce a similar effect may indicate that the process is not one of hydrogen ion catalysis. There is at least a possibility that the acid may act as a specific activator, as it is known that the lipase of castor-oil seed requires activation by an acid, although in this case any acid will suffice.

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BRIEFER ARTICLE

APPLE BLACK ROT (SPHAEROPSIS MALORUM BERK.) IN OREGON[1]

C. R. STILLINGER

While some investigational work was being carried on in the orchards about Corvallis, Oregon, during the fall of 1916, a number of apples with the larger part of their surfaces blackened were noticed hanging upon the trees. In one orchard four large trees of a yellow variety, but apparently seedlings, were found with the greater part of the fruit affected in this way. In fact, both the branches of the trees and the ground underneath were covered with plump as well as shriveled black apples. The general appearance of the affected specimens was so suggestive of black rot that an examination was made for cankers or leaf spots, but none were found.[2] Further search for the disease on apples kept in storage by the Horticultural Department of the Oregon Agricultural College revealed the fact that it was quite common upon the stored apples. The trouble was found upon Northern Spy, Rhode Island Greening, Wagner, Delaware Red, Baldwin, and Newton apples.

Since up to this time the typical black rot of the East had never been reported definitely from the Pacific Coast, an investigation of this disease was made. Previously, Lawrence (1) had reported from western Washington a black spot canker on apple trees, which, although he considered it a possible black rot canker, proved to be the northwestern apple tree anthracnose. Dr. F. D. Heald of the Washington State Agricultural College, Pullman, Washington, in a personal conversation, stated that he had received some apple bark from Vancouver, Washington, during the fall of 1918, infected with what appeared to be black rot, but that he had not been able to investigate the case further.

[1] This work was done in the laboratories of the Botany and Pathology Department of the Oregon Agricultural College, Corvallis, Oregon. The writer wishes to thank Prof. H. P. Barss for his many helpful suggestions and the facilities afforded for carying on the work.

[2] Mr. S. M. Zeller, Department of Pathology, Oregon Agricultural College, Corvallis, Oregon, in a letter dated September 2, 1920, writes that he has collected apple leaf spots this summer at Corvallis, Oregon, and Salem, Oregon, which produce a rot on apples very similar to that produced by a culture of *Physalospora cydonae* secured from Hesler.

In 1915, in an unpublished thesis for the Pathology Department of the University of California, Hahn (2) reported an investigation of an organism that fits the description of the eastern black rot in practically every detail. Because the fungus produced more two-celled than one-celled pycnospores, he finally called it a Diplodia, but could assign it to no particular species. However, the later work of Hesler (3), in 1916, showed that it was not at all unusual for a strain of black rot to produce a majority of two-celled pycnospores. He says: "It appears that certain species of Sphaeropsis have been confused with those of Diplodia. The two genera are separated on the basis of one-celled spores in the former and two-celled spores in the latter. But both genera fail in their chief distinction, so that mycologists have been misled on this point. . . . ." "Apparently, from a single pycnidium in which the spores are of a given type there may develop in succeeding generations a wide variation in size, color, shape, and septation. In an early stage the Macrophoma type may appear in both size and color; later the Eu-Sphaeropsis type, one-celled, brown; and finally the Diplodia forms. The variable shapes that may be found in the generations succeeding a given type indicate that two-celled forms may be mere deviations in the life cycle. . . . ."

"Cultural studies were made of an apple strain (no. 82) with reference to this and other morphological characters. A single ellipsoidal, brown, one-celled pycnospore, 10.8 by 21 μ in size, from a pycnidium in which all the spores were one-celled . . . . . was isolated on March 3, 1913, and a pure culture was developed from it. Examination of the culture at intervals showed the development of hyaline, Macrophoma-like spores. At the end of twenty-four days these had become brownish, and, while the majority were ellipsoidal, averaging from 9 to 10 μ by 21 to 22 μ, a few were pyriform and measured 12.6 by 30.6 μ. At the end of fifty days an occasional spore was found with a cross-wall; after eighty days 30 per cent of the spores were two-celled, and on May 27, 60 per cent were bicellular."

Thus Hesler's experiments showed that the same strain passes through a stage of development from a one-celled, hyaline-greenish to a septate, brownish spore, the type of the spore depending upon its age. Hahn noticed this in his California strain, and based his decision on the older type of spore. Regarding the rot, Hahn states, as a result of his comparative study of the California fungus with a New York and Massachusetts strain of black rot: "This rot is identical with that caused by the Eastern Sphaeropsis, both in appearance and aggressiveness." However, he considered the dark, two-celled, elliptical spore the mature type, and therefore called the fungus a Diplodia. Thus it is very probable that Hahn found the black rot organism in California, but did not recog-

nize it from what was known of the life history of the eastern blackrot at that time.

The strain occurring in Oregon is very probably different from that found by Hahn, since his fungus produces a canker on apple trees, while the Oregon fungus results in a rot of the fruit. The characteristic appearance—the coal black color—makes the Oregon strain easily recognizable wherever the disease may occur. Infected fruit will generally remain longer upon the tree than ripened fruit, even hanging there over winter. In the early stage of the disease, although the apple may be completely infected by the fungus, the fruit remains quite firm and leathery, retaining the original plump appearance of the normal apple. Gradually, however, the apple shrivels and dries into a hard, wrinkled mass, due to the breaking of the cuticle by the fruiting bodies, which permits the rapid evaporation of the moisture from the apple. The surface of the apple turns black up to within a short distance of the advancing margin of the spot invaded by the fungus. Yet, there is generally an area of about 5 mm. bordering the blackened area, which is brownish. The above mentioned fruiting bodies (pycnidia) are scattered quite generally over all the older portion of the blackened surface of the apple.

Internally the fleshy portion of the apple is of a lighter color than the external surface, being brownish black. Here also appears a distinct brown layer separating the healthy from the diseased tissue, which seems to be quite as firm as the healthy portion. The fungus spreads uniformly in all directions in the apple, showing no particular preference for any region. The mycelium penetrates the tissue in the intercellular spaces. It accumulates directly under the cuticle of the apple as a dense, black mass, which gradually enlarges and apparently dissolves its way through the cuticle. At the same time the cells are crushed below, and thus a pocket is formed, filled with a pseudo-paranchymatous mass of hyphae, which gives the structure a reticulate appearance.

As the fungus grows, this pseudo-parenchymatous mass becomes less distinct, and pycnidia develop, which finally rupture the cuticle. These pycnidia are either unilocular or multilocular, and accordingly vary a great deal in size; they sometimes occur singly, but usually in groups of two or three. Inside the pycnidium is a mass of spores held together by a mucous-like substance. This oozes forth in a curled, white, tendril-like cirrhus, which may be 5 mm. long. These spore horns are very conspicuous as they coil out over the surface of the apple. Microscopic examination shows that the mass of spores is held together by a gelatinous substance, which gradually dissolves in water and liberates the spores. After water washes away the spores, a black body remains, appearing very much like a perithecium, with a round ostiolate opening projecting

out of the cuticle, the fragmented edges of which may be seen partially inclosing the structure. Conidiophores are found in the pycnidia. In some cases they consist of single, long, unbranched filaments bearing a spore from the side of each cell; occasionally there is more than one spore produced per cell. In other cases the conidiophores are branched and spores may be scattered along the filaments. In all cases they are sessile and are not aggregated. On the other hand, the spores that occur in the cirrhus are oval to elliptical; hyaline to greenish; generally unicellular, some septate; measuring on an average 9 microns wide by 25 microns in length.

The fungus has been grown upon potato-dextrose agar, Czapek's medium solidified with agar, Czapek's medium as a liquid, and crushed apple tissue. In all of these culture media it grew rapidly and abundantly. The mycelium is appressed to the medium and is hyaline in young cultures, but in older ones it becomes greyish and the substratum black. Thus the fungus produces the same discoloration in culture media that it does in the tissue of the apple. Distinct zonation occurs, the outer zone being hyaline to greyish, the inner and older zones turning greyish black. The optimum temperature for growth is about 20° to 25°C., the fungus being greatly retarded in growth at 15° and killed at 35°C.

After about two weeks, either on solid or liquid media (if the culture is not disturbed), the mycelium collects on the surface in little sclerotia-like masses, which form pycnidia. These develop successively from the center of the culture outward, and are more uniform in size than those on the apple in nature. Eventually a yellowish drop oozes from each pycnidium, resting as a globular body on the top of it. This globular body is composed of thousands of spores held together by a gelatinous substance. The manner of exudation of the spores is somewhat different from that previously described as occurring from the pycnidia on the apple fruit under natural outdoor conditions; it does develop, however, upon inoculated apples that have been kept in a damp chamber. The spore horns that occur in the field have also not been observed in culture. They are evidently reduced to this globular mass, due to the greater amount of moisture present in the air in the cultures. No spores have been found except those that occur in this gelatinous mass or in the spore horns.

The fungus was also grown upon Baldwin and Newton apples. The apples were sterilized with alcohol, washed in sterile water, and placed in a damp chamber. A small section of each apple was raised with a sterilized scalpel and a few spores from a globule of a pycnidium developed in culture were inserted into the apple. In three days the inocu-

lated spots began to turn brown; in eleven days pycnidia were forming upon the apples; and in fourteen days globular exudations began to appear. Within a month the apples were completely blackened.

To determine whether the fungus would infect without a wound through which it might gain entrance, Baldwin and Newton apples were sterilized in 95 per cent alcohol for five minutes, then washed in sterilized water and placed in a damp chamber. Drops of water and drops of Czapek's medium, both sterilized, were placed upon the skins of seven apples, and spores of the fungus from a pure culture were introduced into the drops. Some drops on three other apples were left uninoculated. In five days browning of the spots occurred; in seven, slight blackening was evident; and in twelve, typical pycnidia appeared through the surface of the apples. In all but one case, where the water had either dried up or run off, infection was secured and the typical disease produced. The checks showed no indication of infection. Thus direct penetration by this strain is possible if a small amount of water is present to allow germination. Although Hesler was unable to infect unbroken tissues of the apple fruit with the strains with which he was working, yet this strain seems to be able to do so. Burrill and Blair (4) suggested that this might be possible, while both Scott and Rorer (5) and Brooks and DeMeritt (6) actually demonstrated the penetration of healthy leaf tissue by black rot.

Puncture inoculations upon apple branches, brought into the laboratory during January and placed in large bottles containing water, failed to result in any effect upon the branch other than the injury due to the puncture at the point of inoculation. The inoculated places were covered with celloiden tubes with the ends filled with cotton, one end of the cotton being in contact with water so that the inclosed air in the collar was kept moist. Under these conditions the branches started growth and produced leaves. No inoculation experiments have as yet been made in the field.

To test the viability of the spores, some of the shriveled apples were placed outside in a box near the window, where they would be subject to winter conditions. A few spores that would germinate were found in the pycnidia in the spring.

Since this fungus seemed to agree so well with the published descriptions of the eastern black rot, it was compared with two of the eastern strains in culture and on apples. One was received from Doctor Hesler of Cornell and one from the Bureau of Plant Industry, United States Department of Agriculture. Under the same conditions in tube cultures and plate cultures on the same media no differences could be noted except that this particular Oregon fungus grew somewhat faster, produced a little denser mycelium, and fruited more quickly.

Comparative inoculation studies on Baldwin and Newton apples showed a difference only in rate of growth, there being no variation in the rot produced. The pycnospores produced by the Oregon form were a trifle smaller and had a lighter color than the two eastern strains, which varied somewhat between themselves. Hahn also found considerable variation in the two eastern strains with which he worked, one from New York and one from Massachusetts.

Thus this paper reports for the first time the black rot, *Sphaeropsis malorum*, from Oregon as well as the very probable occurrence of this disease in Washington and California. In none of these states does the disease appear to be of great economic importance as far as observed. The necessity for a more extensive study of the strains of Sphaeropsis and Diplodia has been very much impressed upon the writer as a result of this investigation and he hopes soon to carry on a study of this nature.

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PHYTOPATHOLOGICAL NOTES

Personal. A department of plant pathology has been established at the University of Arizona. Professor J. G. Brown, instructor and assistant professor in the department of biology since 1909, has been appointed head of the new department and plant pathologist of the Agricultural Experiment Station.

Mr. Koichiro Kawakami, assistant in plant pathology of the Botanical Institute of Tokyo University, Japan, succumbed to pneumonia on January 15 of this year. Kawakami's work is for the most part yet unpublished. His work was in the main on the problems of citrus canker, in which he showed great perseverance, patience in obtaining conclusive results, and ingenuity in devising experimental methods. His unpublished work is at present being prepared for presentation by Dr. Tyozaburo Tanaka, formerly of the Bureau of Plant Industry, United States Department of Agriculture, and now residing in Japan.

Kawakami was twenty-nine years old at the time of his death. His education was in the elementary schools of his birthplace, Kisarazu, Chiba prefecture, and in the schools of Tokyo. After graduation from the University of Tokyo he was a fellow of the graduate school of the University where he was fortunate in working under Professor Shirai, chief of the plant pathology division. He later became assistant in plant pathology of the same university. His personality was unusually pleasing, and his interests were deep and sincere. He was an admirer of our American universities and research institutions, and frequently expressed the hope that he would be able to visit the United States for further graduate studies. His death is not only a loss to his friends, his university, and his profession in his own country but is also a loss to the progress of our profession in America and other countries.

H. Atherton Lee,
Philippine Bureau of Science.

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COMPILED BY ALICE C. ATWOOD, AND FLORENCE P. SMITH, BIBLIOGRAPHICAL ASSISTANTS, BUREAU OF PLANT INDUSTRY

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All authors are urged to cooperate in making the list complete by sending their separates and by making corrections and additions, and especially by calling attention to meritorious articles outside of regular journals. Reprints or correspondence should be addressed to Miss E. R. Oberly, Librarian, Bureau of Plant Industry, U. S. Dept. Agric., Washington, D. C.

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